Appendix III

MARKED-UP VERSION OF REWRITTEN PARAGRAPHS

PURSUANT TO 37 C.F.R. § 1.121(b)(1)(iii)

On page 1, please add:

In accordance with the provisions of 35 U.S.C. 120, this application claims the priority and is a continuation-in-part of U.S. Patent Application No. 08/137,032, filed December 15th, 1993, which is a 371 of PCT/GB20/00589, filed April 2nd, 1992, which claims benefit of the priority under 35 U.S.C. 119 of: Great Britain Patent Application No. 91 08386.5, filed April 16, 1991.

On page 7, please rewrite:

Figure 3a and 3b depicts the plasmids (A) pPMM2902 and (B) pBT7-123. The stippled regions represent the CPMV-specific regions of the plasmids with the coding regions being indicated by the wider portions on which the various virus-encoded proteins are marked. Relevant restriction enzyme sites are indicated. Details of the construction of the plasmids are given in Holness, *et al.*, (1989) and Dessens and Lomonossoff (1991).

On page 8, please rewrite:

Figure 4 (SEQ ID NOS:1 and 2) depicts the region of CPMV M RNA which encodes the amino-terminal 40 amino acids of VR23. The numbers below the nucleotide sequence refer to the M RNA sequence and the position of the unique Nhe1 site is indicated. The amino acids involved in forming the βB and βC strands of VP23 are indicated above the amino acid sequence of the protein which is shown using the standard one-letter code.

Figure 5a and 5b depicts (A) the nucleotide sequence (SEQ ID NO:3) of the oligonucleotides used in the construction of pFMDV together with the amino acid sequence (SEQ ID NO:4) encoded by the top(positive) strand and (B) the structure of VP23 after

insertion of the FMDV-specific oligonucleotides (SEQ ID NOS: 5 and 6). The arrowed region indicates the extent of the inserted FMDV epitope. The Nhe1 site not restored during the cloning is indicated by xNhe1. The diagnostic Bgl11 site present in the inserted sequence is also indicated.

Figure 8 (SEQ ID NOS:7 to 10) depicts the construction of a "substitution" vector by site-directed mutagenesis. The asterisk indicates the T residue that is changed to a C by site-directed mutagenesis, thereby creating a novel Aat11 site.

Figure 9a and 9b depicts (A) the nucleotide sequence (SEQ ID NO:11) of the oligonucleotides used in the construction of pMT7-HIV together with the amino acid sequence (SEQ ID NO:12) encoded by the top (positive) strand and (B) the structure of VP23 after insertion of the HIV-specific oligonucleotides (SEQ ID NOS:13 and 14). The arrowed region indicates the extent of the inserted HIV epitope. The diagnostic Pvu1 site present in the inserted sequence is also indicated.

On page 9, please rewrite:

Figure 10a and 10b ddepicts (A) the nucleotide sequence (SEQ ID NO:15) of the oligonucleotides used in the construction of pMT7-HRV together with the amino acid sequence (SEQ ID NO:16) encoded by the top (positive) strand and (B) the structure of VP23 after insertion of the HRV-specific oligonucleotides (SEQ ID NOS:17 and 18). The arrowed region indicates the extent of the inserted HRV epitope. The diagnostic Cla1 site present in the inserted sequence is also indicated.

On page 14, please delete the paragraph beginning on line 1 and ending on line 18, and replace with the following paragraph:

STEP 2. The double-stranded replicative form DNA of M13-JR1 was isolated from infected E. coli strain JM101 cells by the method of Birnboim and Doly (1979). The purified DNA was linearised by digestion with the restriction enzyme Nhe1 and the linearised plasmid treated with calf intestinal phosphatase. The two oligonucleotides with Nhe1-compatible

termini encoding amino acid residues 136 to 160 of VP1 from FMDV were phosphorylated with ATP using polynucleotide kinase and annealed to each other by boiling and slow cooling. The annealed oligonucleotides were ligated into Nhe-1-digested M13-JR1, the ligation mixture used to transform E. coli strain JM101 and the transformation mixture plated out on a lawn of JM101. A large number of plaques were found on the plates, 20 of which were selected for sequence analysis. Bacteriophage were propagated in JM101 and the single-stranded DNA isolated exactly as described by Sanger et al (1980). The nucleotide sequence of the region of the bacteriophage DNA around the Nhe1 site was determined by the dideoxy method as modified by Biggin et al (1983), using an 18mer,5'AGT-TAC-TGC-TGT-AAC-GTC-3' (SEQ ID NO:19), complementary to nucleotides 2735-2752 of the M RNA sequence, as primer. 0f the plaques analysed, one, designated M13-usha1, had a single copy of the desired sequence in the correct orientation.

On page 17, please delete the paragraph beginning on line 9 and ending on line 15, and replace with the following paragraph:

1. 1st strand cDNA to purified CPMV M RNA was synthesised exactly as described by Lomonossoff et al (1982), using pdT_{12-18} as a primer. 2nd strand synthesis was primed using the following oligonucleotide:

PstI T7 Promoter ↓ 5' end M RNA

5' CCCTGCAGTAATACGACTCACTATAGTATTAAAATCTTAATAG (SEQ ID NO:20)

Conditions for synthesis were as described in Lomonossoff et al (1982) and Shanks et al (1986).

On page 21, please delete the paragraph beginning on line 21 and ending on line 29, and replace with the following paragraph:

STEP 1. M13-JR-1 (see Figure 6) was propagated in E. coli strain CJ236 and dU-containing single-stranded DNA isolated as described by Kunkel (1985). The T to C mutation at position 2740 of the M RNA sequence was made by oligonucleotide-directed mutagenesis of dU-containing single-stranded M13-JR1 DNA using the primer CTG-CTG-TGA-CGT-CTG-AAA-A (SEQ ID NO:21) as described by Kunkel (1985). This

resulted in the construction of clone M13-JRAat11. The mutation was confirmed by dideoxy sequence analysis of single-stranded DNA (Biggin et al, 1983) and by restriction enzyme digestion of the double-stranded replication form DNA.

On page 25, please delete the paragraph beginning on line 2 and ending on line 26, and replace with the following paragraph:

pMT7-FMDV-1: To demonstrate that modified viral capsid proteins were synthesised in the inoculated leaves of the group 4 plants, samples of the frozen leaf tissue were finely ground and extracted with 1X Laemmli sample buffer. The extracts were electrophoresed on 15% polyacrylamide-SDS gels and the proteins transferred to nitrocellulose membranes using a Biorad semi-dry transfer cell. The membranes were probed either with serum raised against whole CPMV virus particles or with a serum raised against the synthetic oligopeptide, VPNLRGDLQVLAQKVARTLP(CG) (SEQ ID NO:22), corresponding to residues 141-160 of VP1 of FMDV strain O_1. This sequence corresponds to the epitope which was inserted into VP23 in pMT7-FMDV. Both antisera were raised in rabbits. Western blot analysis was carried out using alkaline phosphatase-conjugated goat anti-rabbit IgG as the second antibody. The protein extracts of all five group 4 plants were found to react with the anti-CPMV serum indicating that the virus coat proteins were synthesised in the inoculated leaves of the group 4 plants. When similar blots were probed with the anti-FMDV oligopeptide serum, a single band lit up in the extracts from each of the group 4 plants (Figure 11). This band migrated with an apparent molecular weight of 24kDa, which is exactly the size expected for VP23 carrying the FMDV loop. No product of similar size could be seen when extracts from mock-inoculated or wild-type CPMV-inoculated leaves were analysed (Figure 11). Likewise, purified wild-type CPMV coat proteins did react with the FMDV-specific antiserum. Furthermore, pre-treatment of the anti-FMDV serum with the peptide which was used to raise it, abolished the reaction with the extracts from the group 4 plants demonstrating the specificity of the immunological reaction. These results demonstrate that the inoculated leaves of the group 4 plants contained CPMV coat proteins harbouring the FMDV loop.